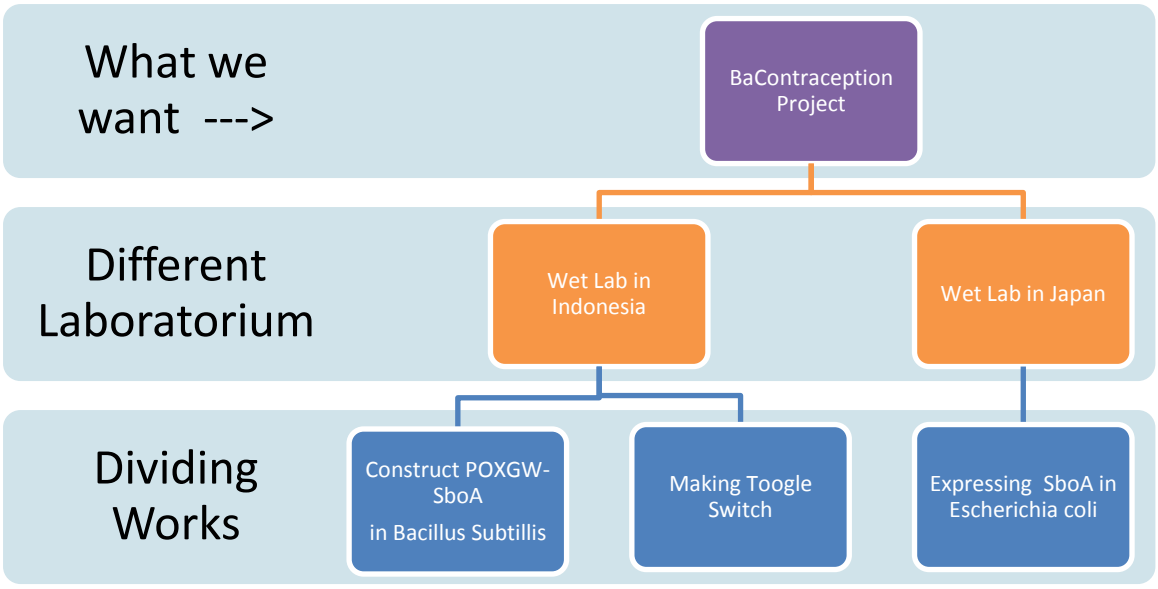
RESEARCH LOGBOOK

BACONTRACEPTION UNIVERSITAS INDONESIA

Introduction

We devide our work into two works for wet laboratory. These are wet laboratory in one laboratorium in Japan and two laboratorium in Indonesia. The practician in Indonesia, especially work for constructing SboA and toogle switch in Bacillus subtilis wet lab are Jessica Luis, Faudina Fitra, Adi Nugroho Harlianto, and I Gusti Ngurah Raka. Otherwise, The Practician in Japan, especially work for constructing sboA expression in escherichia coli, is Yudhistira Oktaviandi. Our purpose is expressing contraception in Bacillus subtillis, but we also do some work in Escherichia coli because we wanto to get more data to support our project (eg. the veasiblity SboA in Eschericia coli). First, we will give short explanation how we divide works to make bacontraception. This explanation is given by mind map at below.



The different work in both laboratorium (Japan and Indonesia) is abosolutely affecting our logbook. That's why, we make two part of our logbook.

Understanding Our Logbook

- We have been explained the protocol of bacontraception project which contain information about activity, general information ingredients and tools we use, so we

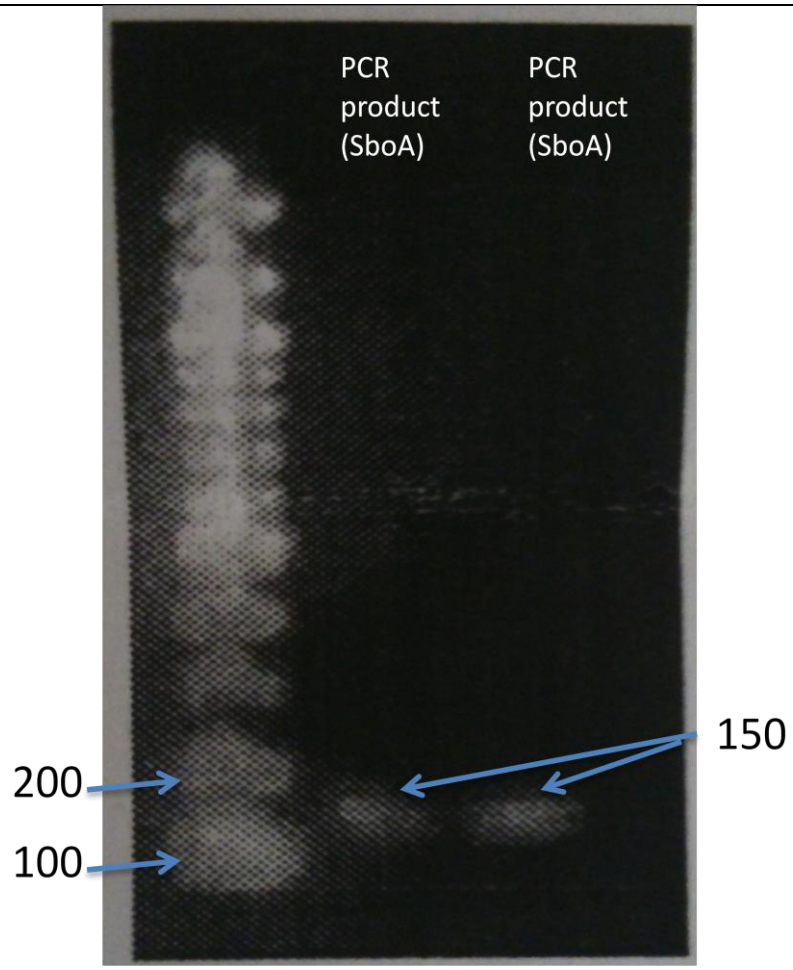
just give other information in this logbook, these are tittle of work (the reader can read our protocol and step of work from 'protocol page'), date of work, the practician who do this work (we usually use the keyword name of practician), the result of work, and more adjusment information (e.g the detailed amount of ingredients we use).

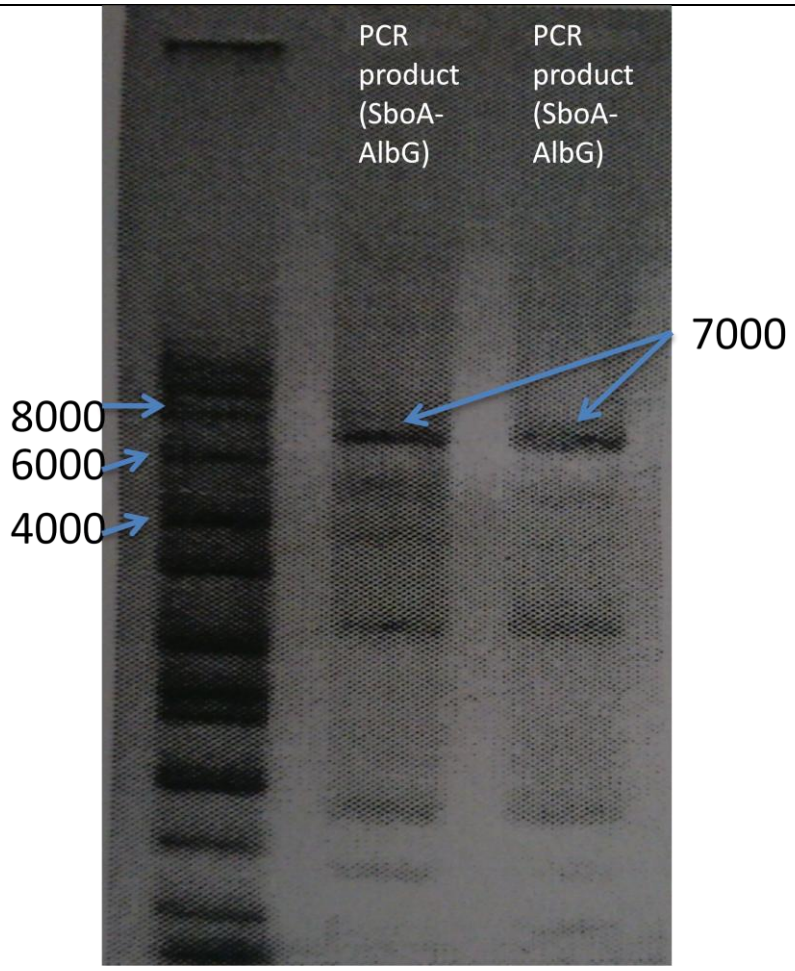
- The keyword name of practician :
  - Jessica Luis (J)
  - Adi Nugroho Harlianto (A)
  - I Gusti Ngurah Raka (R)
  - Faudina Nurilla Fitra (F)
  - Yudhistira Oktaviandie (Y)

Part One : Insertion of SboA gene and SboA-AlbG gene into E coli DH5alpha using pET9a

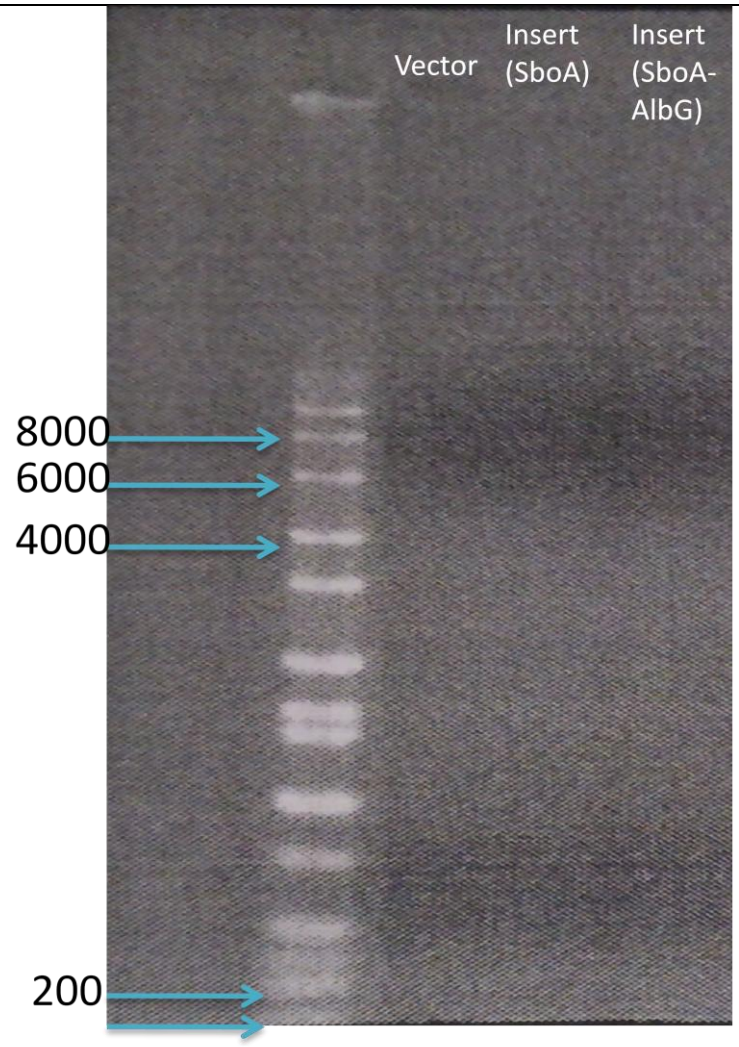
The practian is Yudhistira Oktaviandi (Y)

Date	No.	Activity
24/06/2015 (Wednesday)	1.	Re-suspending Synthetic Dried DNA.
	2.	Preparing Agarose Agar 1%
25/06/2015 (Thursday)	1.	Amplification of SboA & SboA-AlbG by PCR.
	2.	PCR Product Purification (SboA gene)
	3.	Checking PCR purified product by agarose electrophoresis (SboA gene)

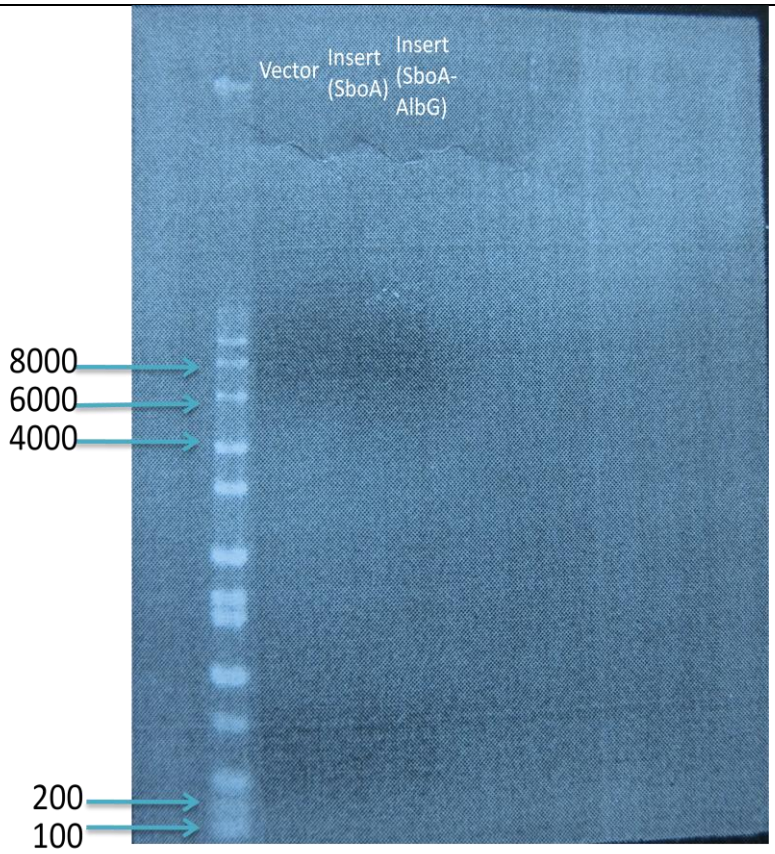
		
26/06/2015 (Friday)	1.	Checking PCR purified product by aga//rose electrophoresis (SboA-AlbG gene)

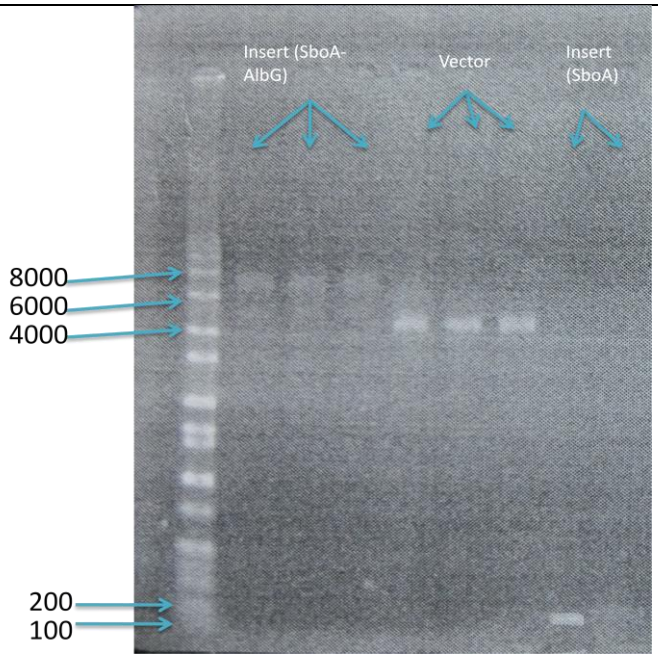
		
	2.	Agarose Gel DNA Purification for PCR product (SboA-AlbG gene)
29/06/2015 (Monday)	1.	Culture E coli DH5alpha in LB broth
	2.	Checking purified PCR product concentration by NanoDrop (ng/µL) : Purified SboA gene Sample 1 : 20.8 Purified SboA gene Sample 2 : 15.9 Purified SboA-AlbG gene Sample 1 : 62.1 Purified SboA-AlbG gene Sample 2 : 115 *SboA gene has low concentration because using too much glass beads (5 µL for 25 µL PCR products)*
30/06/2015 (Tuesday)	1.	Plasmid Isolation (pET9a) from cultured E coli DH5α
	2.	Checking isolated plasmid (pET9a) concentration by NanoDrop (ng/µL) : Purified pET9a Sample 1 : 272.1 Purified pET9a Sample 2 : 196.4

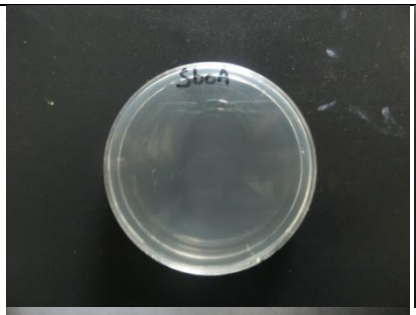
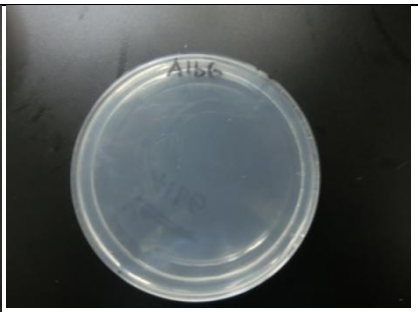
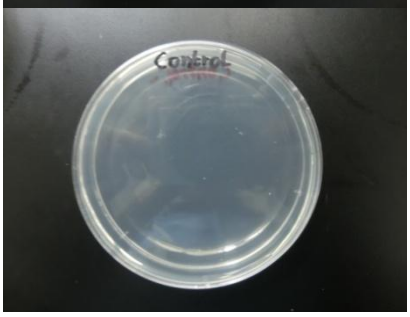
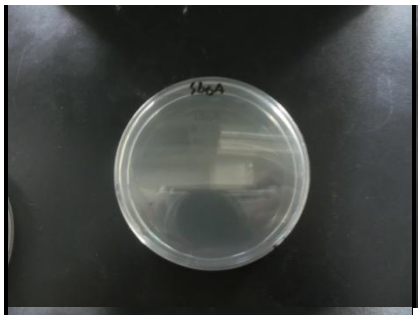
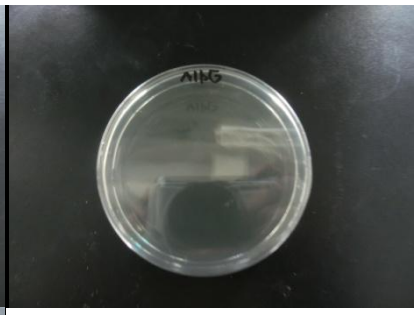
01/07/2015 (Wednesday)	1.	Double Digest PCR-purified product and vector DNA : 100 ng CutSmart Buffer : 0.5 µL Restriction Enzyme : 2 x 0.2 µL (NdeI & BamHI) dH_2O : 3.1 µL Incubate 2 hour at 37^oC
	2.	Agarose gel DNA purification for digested DNA *No band appear when see using transilluminator/GelNinja because reaction volume very small*
02/07/2015 (Thursday)	1.	Double Digest PCR-purified product and vector DNA : 900 ng CutSmart Buffer : 2 µL Restriction Enzyme : 2 x 0.5 µL (NdeI & BamHI) dH_2O : until volume reaction 20 µL in total Incubate in 37^oC static incubator for minimum overnight (16-20 hr)
03/07/2015 (Friday)	1.	Agarose gel DNA purification for digested DNA *Band appears clearly in transilluminator/GelNinja*
	2.	Checking purified-digested DNA by agarose electrophoresis

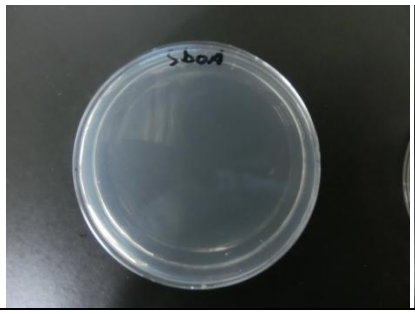
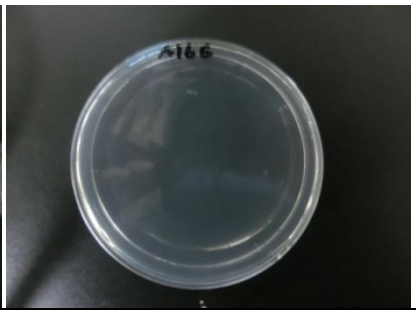
		 *No band appear, there's something wrong when agarose gel DNA purification*
06/07/2015 (Monday)	1.	Double Digest PCR-purified product and vector DNA : 500 ng CutSmart Buffer : 5 µL Restriction Enzyme : 2 x 0.25 µL (NdeI & BamHI) dH_2O : until volume reaction 20 µL in total Incubate in 37^{o}C static incubator for 1 hour *This composition was following NEB digestion protocol (Quantitative)*
	2.	Agarose gel DNA purification for digested DNA *Glass beads was decreased from 5 µL to 3 µL. Band appears clearly in transilluminator/GelNinja*
	3.	Checking purified-digested DNA by agarose electrophoresis

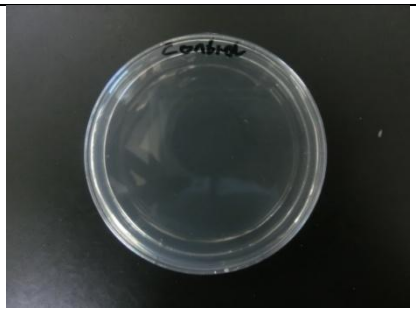
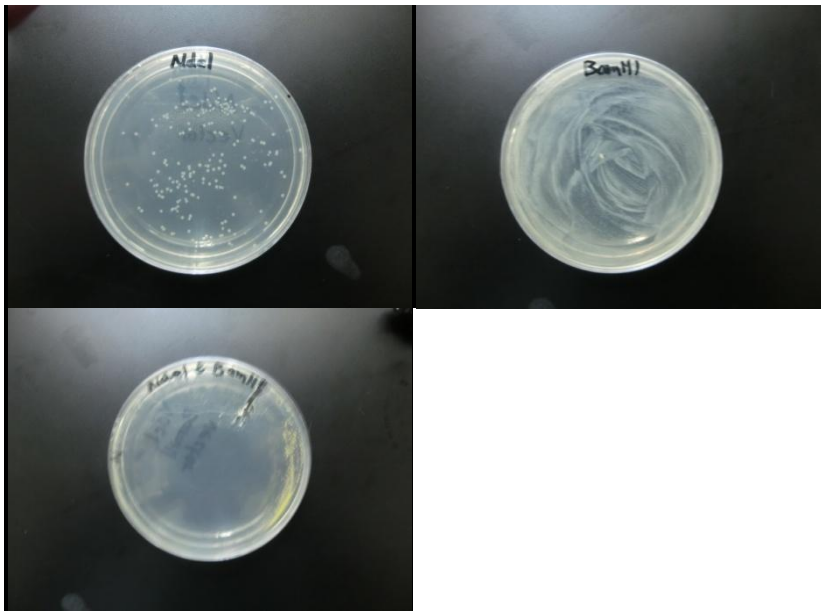
		No band appear
07/07/2015 (Tuesday)	1.	Double Digest PCR-purified product and vector DNA : 500 ng CutSmart Buffer : 5 µL Restriction Enzyme : 2 x 0.25 µL (NdeI & BamHI) dH_2O : until volume reaction 20 µL in total Incubate in 37^oC static incubator for 1 hour
	2.	Agarose gel DNA purification for digested DNA *Band appears clearly in transilluminator/GelNinja*
	3.	Checking purified-digested DNA by agarose electrophoresis *No band appear*
08/07/2015 (Wednesday)	1.	Amplification of SboA & SboA-AlbG by PCR.
	2.	PCR Product Purification (SboA gene)
	3.	Agarose Gel DNA Purification for PCR product (SboA-AlbG gene)
	4.	Checking purified PCR product concentration by NanoDrop (ng/µL) : Purified SboA gene : 86.3 Purified SboA-AlbG gene : 109.5
09/07/2015 (Thursday)	1.	Double Digest PCR-purified product and vector DNA : 1000 ng CutSmart Buffer : 5 µL Restriction Enzyme : 2 x 0.5 µL (NdeI & BamHI) dH_2O : until volume reaction 20 µL in total Incubate in 37^oC static incubator for 1 hour *This composition was following NEB digestion protocol (Quantitative) and the amount of digested DNA increased*
	2.	Agarose gel DNA purification for digested DNA *Band appears clearly in transilluminator/GelNinja*
	3.	Checking purified-digested DNA by agarose electrophoresis *No band appear even though amount of DNA had been increased*

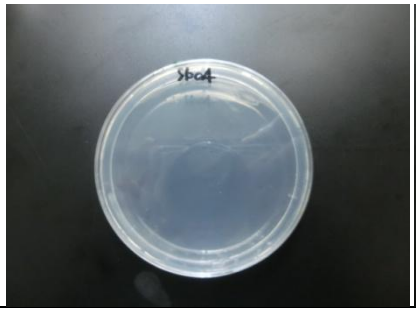
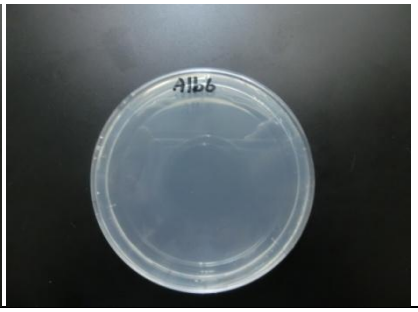
		
10/07/2015 (Friday)	1.	Double Digest PCR-purified product and vector DNA : 900 ng CutSmart Buffer : 2 µL Restriction Enzyme : 2 x 0.5 µL (NdeI & BamHI) dH_2O : until mixture volume 20 µL in total Incubate in 37^oC static incubator for minimum overnight (16-20 hr)
11/07/2015 (Saturday)	1.	Move digestion mixture into freezer
14/02/2015 (Tuesday)	1.	Agarose gel DNA purification for digested DNA *Band appears clearly in transilluminator/GelNinja*
	2.	Checking purified-digested DNA by agarose electrophoresis

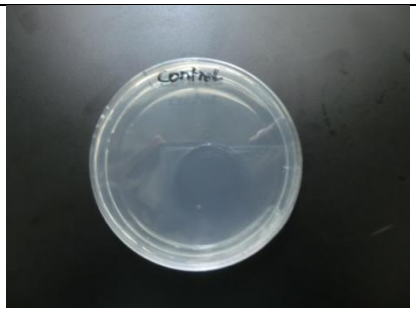
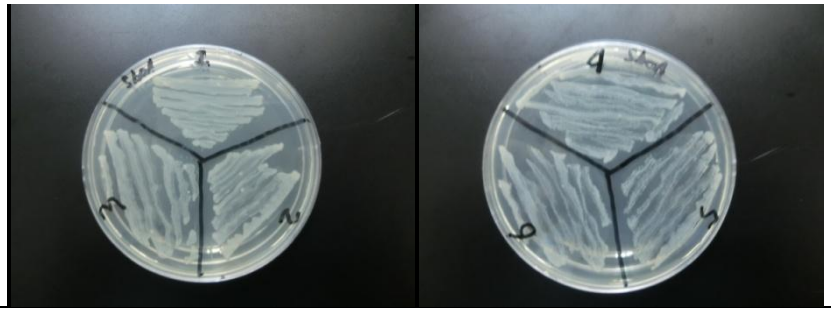
		 *Drowning agar in EtBr for 30 minutes and increasing amount of DNA for checking by electrophoresis from 1 µL to 3 µL, finally band appears. So maybe it's not problem within digestion or purification but problem in checking by electrophoresis*
15/07/2015 (Wednesday)	1.	Ligation using Takara Ligation Kit 1. Insert (SboA gene) : 2 µL Vector : 0.5 µL Takara Ligation mix : 2.5 µL 2. Insert (SboA-AlbG gene) : 2.2 µL Vector : 0.3 µL Takara Ligation mix : 2.5 µL Incubate at 16 ^{o}C for 30 minutes
	2.	Checking purified PCR product concentration by NanoDrop (ng/µL) : Purified digested SboA gene : 38.6 Purified digested SboA-AlbG gene : 102 Purified digested plasmid : 67
	3.	Transformation
16/07/2015 (Thursday)	1.	Move plate from incubator and check for colonies.

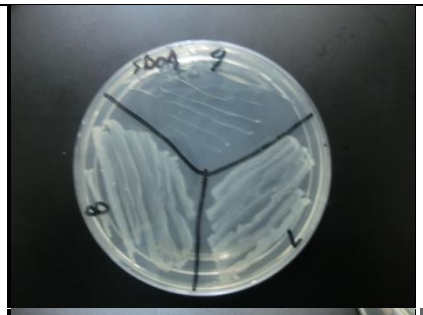
		   *No colony grow; next ligation and transformation using T4 ligase (NEB products)*
21/07/2015 (Tuesday)	1.	Ligation using T4 ligase Insert: 3 µL (SboA & SboA-AlbG in different tube) Vector: 1 µL T4 ligase buffer: 2 µL dH_2O: 13 µL T4 ligase: 1 µL Incubate for 6 hours at 16 ^oC
	2.	Transformation
22/07/2015 (Wednesday)	1.	Move plate from incubator and check for colonies.   

		No colony grow; composition of ligation mixture wasn't appropiate, next ligation will use quantitative method as suggested as NEB ligation protocol
23/07/2015 (Thursday)	1.	Ligation using T4 ligase Insert: 6.5 ng (SboA gene) Vector: 67 ng T4 ligase buffer: 2 µL dH_2O: until mixture volume 20 µL T4 ligase: 1 µL Insert: 351.75 ng (SboA – AlbG gene) Vector: 67 ng T4 ligase buffer: 2 µL dH_2O: until mixture volume 20 µL T4 ligase: 1 µL Incubate for 16 - 20 hours at 16 ^oC
	2.	Culture *E coli* DH5alpha in LB broth
24/07/2015 (Friday)	1.	Transformation
	2.	Plasmid Isolation (pET9a) from cultured E coli DH5α
	3.	Digest pET9a with 3 different treatment in 3 different plate : 1. Digest using NdeI only with no insert 2. Digest using BamHI only with no insert 3. Digest using NdeI & BamHI with no insert
	4.	Agarose purification of digested pET9a
25/07/2015 (Saturday)	1.	Move plate from incubator and check for colonies.  

		 *No colony grow*
27/07/2015 (Monday)	1.	Ligation of digested pET9a
28/07/2015 (Tuesday)	1.	Transformation
29/07/2015 (Wednesday)	1.	Move plate from incubator and check for colonies  *2 plates have colonies inside which cut by NdeI only and BamHI only; for other plate which cut by NdeI & BamHI, there's no colony. It could be conclude that nothing going wrong with digestion and ligation.*
03/08/2015 (Monday)	1.	Amplification of SboA gene & SboA-AlbG gene
	2.	Purification of SboA gene & SboA-AlbG gene
	3.	Checking purified PCR product concentration by NanoDrop (ng/µL) : Purified SboA gene: 119.8 Purified SboA-AlbG gene: 100
04/08/2015 (Tuesday)	1.	Double Digest PCR-purified product and vector DNA : 1000 ng

		CutSmart Buffer : 5 µL Restriction Enzyme : 2 x 0.5 µL (NdeI & BamHI) dH_2O : until volume reaction 20 µL in total Incubate in 37^oC static incubator for 1 hour
	2.	Purification of digested SboA gene, SboA-AlbG gene & pET9a Purified pET9a : 130.3 ng/ µL *Only pET9a successfully purified*
	3.	Ligation Insert (SboA gene) : 5.5 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : 11.89 µL Insert (SboA-AlbG gene) : 262.5 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : 11.89 µL Incubate for 16 - 20 hours at 16 ^oC
05/08/2015 (Wednesday)	1.	Move ligation product into -30 ^oC freezer
	2.	Transformation
06/08/2015 (Thursday)	1.	Move plate from incubator and check for colonies  

		 *No colony grow*
07/08/2015 (Friday)	1.	Ligation Insert (SboA gene) : 5.5 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : 11.89 µL Insert (SboA-AlbG gene) : 262.5 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : 11.89 µL Incubate for 16 - 20 hours at 16 ^oC
08/08/2015 (Saturday)	1.	Move ligation product into -30 ^oC freezer
10/08/2015 (Monday)	1.	Transformation *Decreasing heat shock temperature to 40 ^oC*
11/08/2015 (Tuesday)	1.	Move plate from incubator and check for colonies *9 colonies grow in SboA plate; 6 colonies grow in SboA-AlbG plate*
	2.	Replicate colonies
12/08/2015 (Wednesday)	1.	Move replica to -20 ^oC freezer 

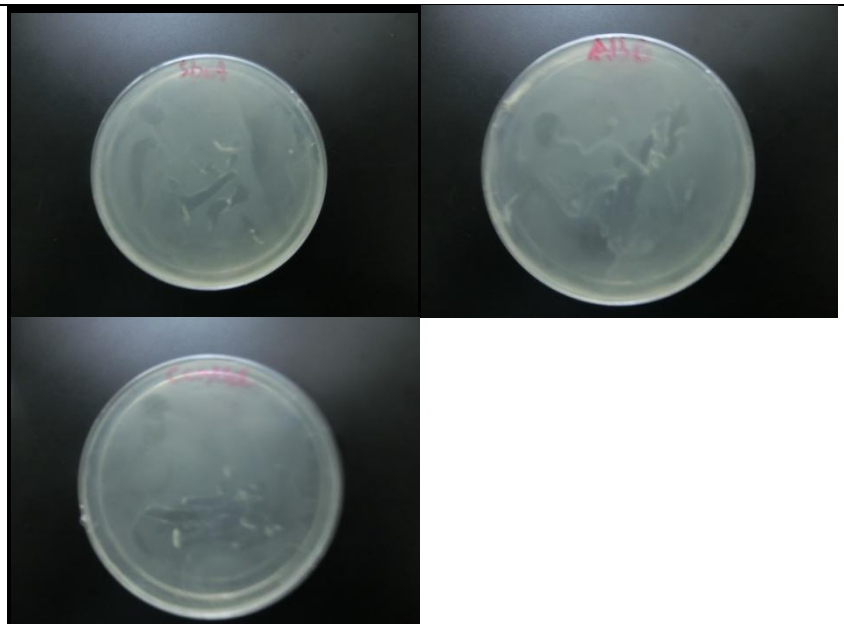


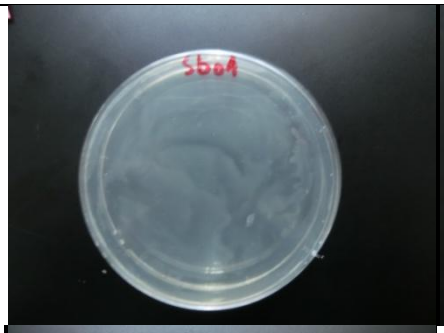
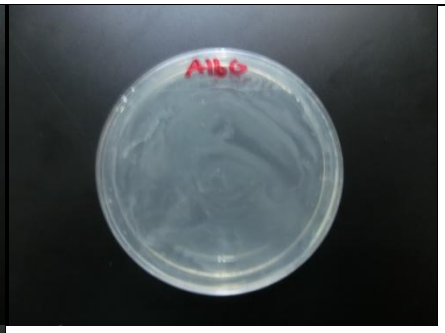
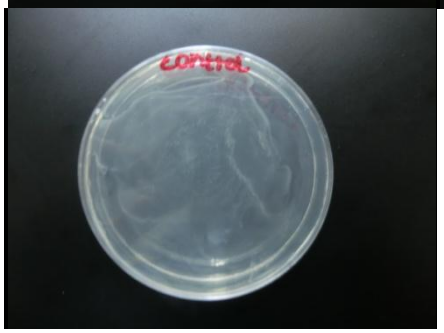
Result: After transformation of SboA gene and SboA-AlbG gene using pET9a, there are some colonies grow, but it hasn't check yet.

Name : Yudisthira Oktaviandie

Project : Insertion of SboA gene and SboA-AlbG gene into shuttle vector PTRKH3-ldhGFP

03/08/2015 (Monday)	1.	Amplification of SboA gene & SboA-AlbG gene
	2.	Purification of SboA gene & SboA-AlbG gene
	3.	Culture bacteria containing PTRKH3-ldhGFP
04/08/2015 (Tuesday)	1.	Isolation of PTRKH3-ldhGFP
	2.	Double Digest PCR-purified product and vector DNA : 1000 ng CutSmart Buffer : 5 µL Restriction Enzyme : 2 x 0.5 µL (NdeI & BamHI) dH_2O : until volume reaction 20 µL in total Incubate in 37^oC static incubator for 1 hour
	3.	Purification of digested SboA gene, SboA-AlbG gene & PTRKH3-ldhGFP
	4.	Ligation Insert (SboA gene) : 2.8 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : until 20 µL Insert (SboA-AlbG gene) : 131.25 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : until 20 µL Incubate for 16 - 20 hours at 16 ^oC
05/08/2015 (Wednesday)	1.	Move ligation product into -30 ^oC freezer
	2.	Transformation
06/08/2015 (Thursday)	1.	Move plate from incubator and check for colonies

		 *Bacteria grow as a smear not a dot (single colony)*
07/08/2015 (Friday)	1.	Ligation Insert (SboA gene) : 2.8 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : until 20 µL Insert (SboA-AlbG gene) : 131.25 ng T4 Ligase Buffer : 2 µL T4 DNA Ligase : 1 µL Vector : 50 ng dH_2O : until 20 µL Incubate for 16 - 20 hours at 16 ^oC
08/08/2015 (Saturday)	1.	Move ligation product into -30 ^oC freezer
10/08/2015 (Monday)	1.	Transformation
11/08/2015	1.	Move plate from incubator and check for colonies

(Tuesday)		   *Bacteria grow as a smear not a dot (single colony); there is some problem with MIC of antibiotics that we made. We're using erithromycin tablet instead of liquid erithromycin. Next time, we'll use liquid erithromycin to get adequate MIC of antibiotics.*
	2.	Isolation of plasmid from smear colonies
	3.	Check plasmid by digestion and run on agarose electrophoresis *From four plate we test, only plate 3 that contain plasmid.*

Result: Bacteria grow as a smear because of wrong concentration of antibiotics. From four plate, only one plate (plate 3) that contains plasmid and it hasn't check yet.

Part Two : Insertion of SboA gene and SboA-AlbG gene into E coli DH5alpha using pET9a

Date	Number	Activity	Result	Detailed Information
06/05/2015 Wednesday	1.	Making Nutrient Broth and Nutrient Agar (**J)**		
	2.	Streaking Bacillus Subtillis		We incubate

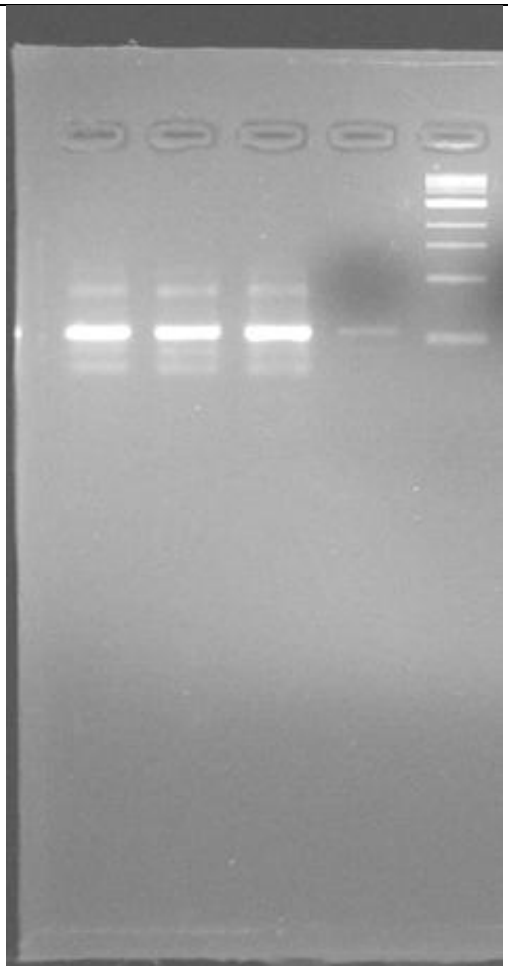
		from Frozen Stock (**F)**		Bacillus subtilis from frozen stcock at incubator 32Oc We made 120 ml nutrient agar (2,76 grams) and 50 ml Nutrient Broth (0,4 grams)
07/05/2015 Thursday	1.	Genom Isolation from Bacillus subtilis **(F and A)**		
	2.	Ethium bromide electrophoresis **(F and A)**		
11/05/2015 Monday	1.	Making Mrs Broth and Mrs Agar (F)		
13/05/2015 Wednesday	1.	Isolation Lactobacillus Fermentum and Lactobacillus		

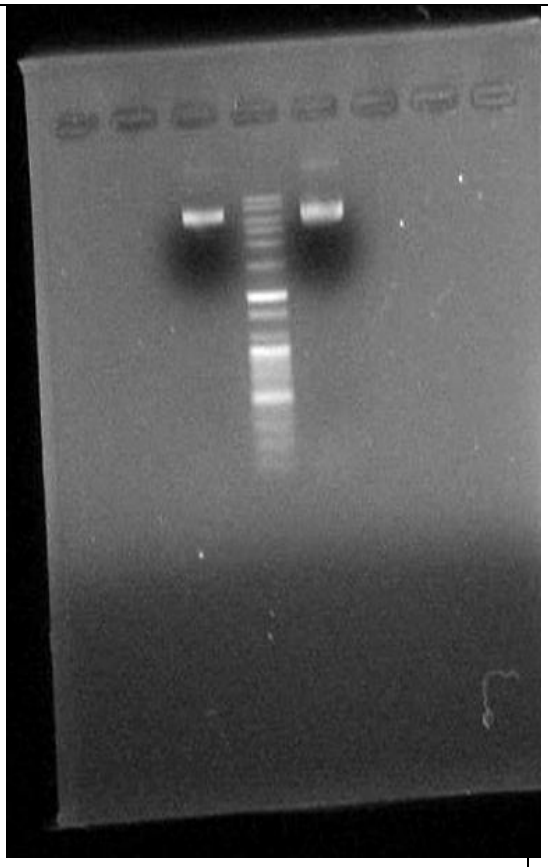
		Plantarum **(R)**		
	2.	Ethibium bromide electrophoresis Bromide for the result of isolation lactobacillus fermentum and plantarum**.(R)**		
14/05/2015 Friday	1.	Polimerase Chain Reaction with Taq Polymerase for L.plantarum, L.Fermentum, and Bacillus subtilis **(A)**		
	2.	Ethibium bromide electrophoresis for PCR confirmation (A)		
19/05/2015 Wednesday	1.	Doing PCR again for L.plantarum, L.Fermentum, and Bacillus subtilis. **(F)**		
	2.	Ethibium bromide electrophoresis for PCR confirmation**(R)**		
6/07/2015 Monday	1.	Inokulasikan bac A and Bac B in Nutrient Broth (R)		
	2.	Isolation plasmid		

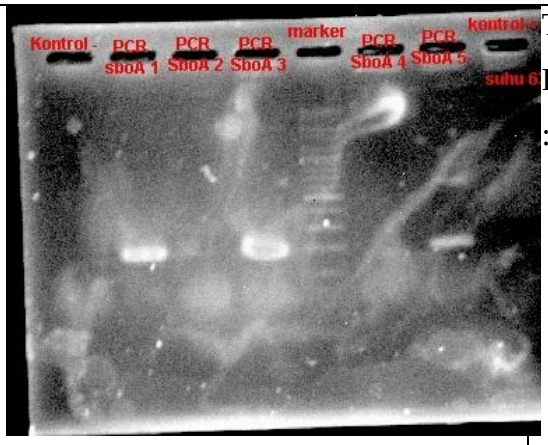
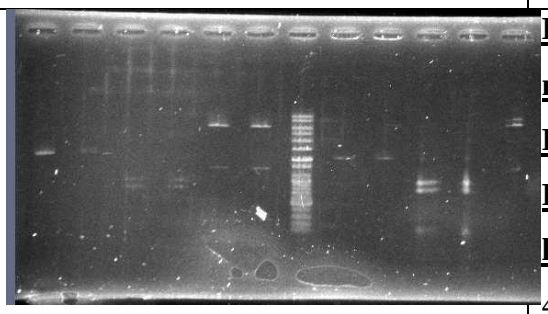
		Bac A and Bac B (R)		
	3.	Ethibium bromide electrophoresis for Isolation confirmation (A)		
07/07/2015 Tuesday	**1.**	Streaking bacillus subtilis in Nutrient Agar and Lactobacillus fermentum and Lactobacillus plantarum in agar (J)		
	2.	Preparing materials for gateway (R)		
08/07/2015 Wednesday	1.	Gateway sboA (A)		
	2.	Primer gateway dilution (A)		
	3.	PCR gateway		
09/07/2015 Thursday	1.	Making Escherichia coli DH5alpha competent cell (J)		
	2.	Transformation BP into Escherichia coli DH5alpha (R)		
	3.	Incubate Escherichia coli containing BP		

		clonase (R)		
10/07/2015 Friday	1.	Making Escherichia coli DH5alpha competent cell (R)		
	2.	Transformatiom LR clonase into Escherichia coli DH5alpha competent cell (R)		
	3.	Incubate overnight the result of LR clonase transformation (R).		
23/7/2015	1.	Replica plating POXGW transformation in Eschericia coli DH5alpha (F)		

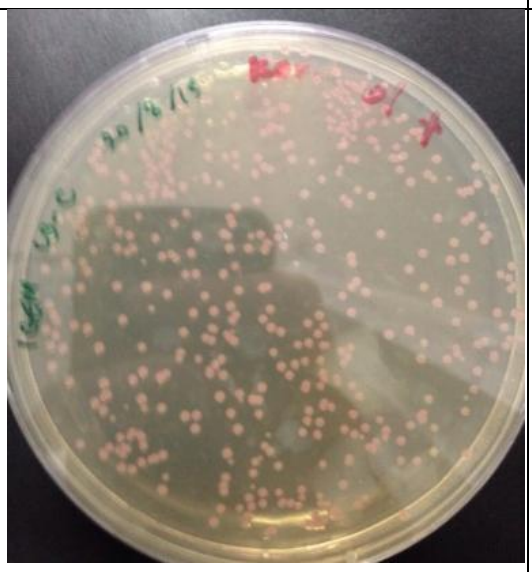
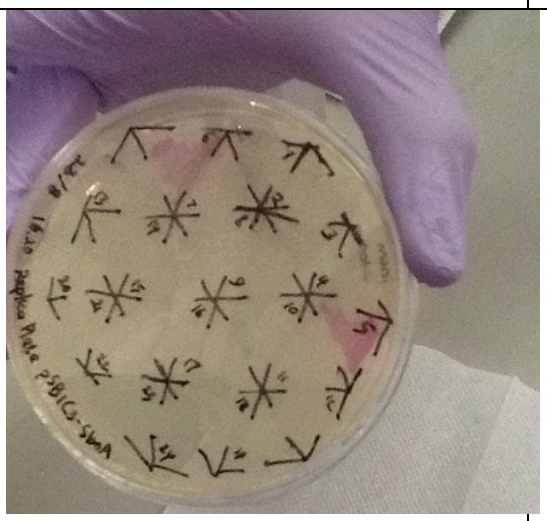
24/07/2015	1.	Checking Replica plating POXGW (R)		
5/08/2015	1.	Transform PSB1C3 into Top 10Escherichia coli (J)		
	2.	Streak bacillus subtilis overnight (A)		

11/08/2015 Saturday	1.	Isolation Escherichia coli containing BP clonase and electrophoresis isolation BP (J)		
	2.	PCR confiration using Pdonr (A)		

	3.	Isolation Escherichia coli containing LR clonase and electrophoresis LR clonase (R)		
	4.	Isolation Escherichia coli containing pOXGW (J)		
	5.	Replica plating for LR. BP, and POXGW. (J)		
12/08/2015 Sunday	1.	Isolation from all replica plating (J)		
15/08/2015 Friday	1.	Making Bacillus subtilis competent cell (R)		
	2.	Electroporasi bacillus subtilis		

18/08/2015 Tuesday	1.	PCR sboA-taq (R)		Total Reaction : 20ul
19/08/2015 Wednesday	1.	Purification the result of PCR sboA-taq using Qiaex (J)		
	2.	Digestion PSB1C3 linier and sircular a (F)		**<u>Digestion Plasmid PSB1C3 linier :</u>** 4 ul PSB1C3 plasmid linier and 4 ul Enzym master mix
	3.	Digestion POXGW (F)		Weight amount of POXGW : 184 ng/ul, we use 5 ul

				(1 ug) BSA 2,5 ul, Buffer orange bioneer for digestion : 2,5 ul, XhoI and SpH I : 0,05 ul (each enzym restrictio n. Total reaction 25 ul
20/08/20 15 Thursda y	1.	Digestion SboA-Taq without heat kill (F)		
22/08/20 15 Saturday	1.	Making E.coli competent cell (J))		
	2.	Heat Kill PSB1C3-SboA digestion on 20 /08/2015 (A)		
	3.	Transform PSB1C3-SboA into E.coli competent cell.		

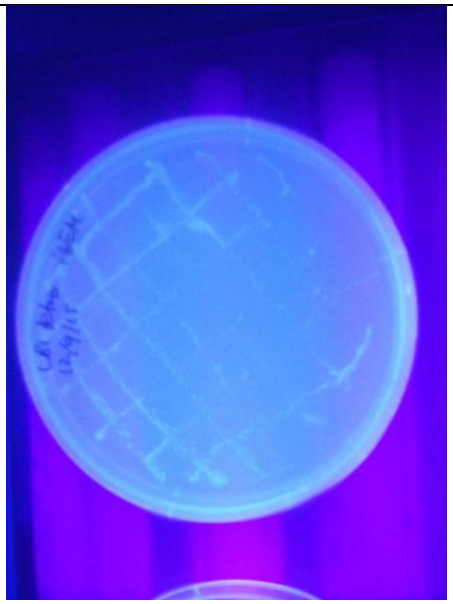
		Then, incubate overnight (A)		
	4.	Isolation using High Speed Qiagen (J)		
23/08/2015 Sunday	1.	Check the PSB1C3-SboA transformation on 22/08/2015 (A)		
	2.	Replica plating PSB1C3-SboA transformation and Incubate overnight (A)		
25/08/2015 Tuesday	1.	PCR Accu Power Premix (R)		
26/08/2015 Wednesday	1.	Digestion PSB1C3-SboA using Pst 1 and EcoR1 (F)		
	2.	Nanodrop		

31/08/2015 Monday	1.	Incubate PSB1C3-SboA (F)		
	2.	PCR confirmation PSB1C3-SboA (from isolation on 22/08/2015)		
	3.	Electrophoresis PCR confirmation PSB1C3-SboA sample (J)		
	4.	Digest PSB1C3-SboA (F)		
01/09/2015 Tuesday	1.	Inoculate plasmid from replica plating into Luria Broth (F)		
	2.	Digestion using Cutsmart, but it hadn't be incubated in incubator 37Oc (F)		Using 25O nanograms plasmid
02/09/2015 Wednesday	1.	Electrophoresis the result of PCR and digestion on 31 August 2015 and on 01 September 2015 (A)		
09/09/2015 Wednesday	1.	Drying DNA overnight		
	2.	Digestion POXGW (F)		

	3.	Purification plasmid (J)		
	4.	Electrophoresis PCR and digestion POXGW (A)		
10/09/2015 Thursday	1.	Plasmid containing POXGW-SboA sent to IGEM (A)		
	2.	Electrophoresis Purification plasmid on 09 September 2015 (A)		
11/09/2015 Friday	1.	Preparing material for induction (A)		
	2.	Preparing 12 plate filled Luria Broth and kanamycin for transformation (A)		
	3.	Making Luria Broth containing kanamycin		
	4.	TransformationPET9A into BL21CP. Then, incubate overnight (A)		
	5.	Ligation toogle switch with front fragment		

		"POXGW" and fragment "PSB1C3". Then, incubate at room temperature overnight. (A)		
12/09/2015 Saturday	1.	Checking the plate containing transformation BL21CP (R)		
	2.	Making BL21CP competent cell (R)		
	3.	Transformation BL21 again (A)		
	4.	Gibson Assembly between POXGW and PSB1C3 (A)		
13/09/2015 Sunday	1.	Incubate 3-4 hours BL21CP in incubation shaker for transformation.		
	2.	Transform gibson into BL21CP (A)		
14/09/2015 Monday	1.	Purification SboA using Qiaquick (J)		
	2.	Double digestion Poxgw using XhoI an Sph 1 as enzym restriction (F)		
	3.	Double digestion pSB1C3 linear and sircular using		

		EcoRI HF and PstI (F)		
	4.	Purification the result of digestion (POXGW and PSB1C3) (J)		
	5.	Agarose gel Electrophoresis for confirmation (J)		
14/09/2015 Tuesday	1.	Making Bacillus subtilis competent cell (R)		
	2.	Transformation into Bacillus subtilis competent cell (R)		
	3.	Electrophorasi Bacillus subtilis 168 (R)		
	4.	Agarose gel Electrophoresis again for confirmation the result of purification (yesterday)		
	5.	Re- Double digestion pSB1C3 linear and sircular using EcoRI HF and PstI (F)		
	6.	Purification the result from double		

		digestion psB1C3 linear and sircular (J)		
	7.	Agarose gel Electrophoresis for confirmation (J)		
15/09/2015 Wednesday	1.	Ligation POXGW and SboA (F)		
	2.	Inoculate single coloni e.coli top 10 into luria broth then culture overnight/Preparing for competent cell on Saturday (F)		
17/09/2015 Friday	1.	Transform the ligation POXGW and SboA into E.coli (Y)		
	2.	Replica plating		
18/10/2015	1.	Running SDS page (A)		

	2.	Isolation PSB1C3-Toogleswitch		
	3.	Well difussion assay		